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(54) Title: LIPOPHILIC ACYLPYRAZINAMIDE PRODRUGS

(57) Abstract

Lipophilic acylpyrazinamide prodrugs which are effective against intracellular pathogens. These prodrugs are especially useful against members of the genus *Mycobacterium* and can be utilized in targeted delivery systems, especially liposomes.

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LIPOPHILIC ACYLPYRAZINAMIDE PRODRUGS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to pharmaceutical compounds and, more particularly, to lipophilic pyrazinamide prodrugs, which possess antimicrobial activity as well as compositions containing these prodrugs and methods of use.

DESCRIPTION OF RELATED ART

Microorganisms have adopted a wide variety of strategies which enable them to colonize a host. Among those organisms which are pathogenic those which are capable of causing or residing in the intracellular milieu are among those which are most difficult to treat. Such organisms are of a wide variety and include bacteria, protozoa, fungi, and viruses.

A typical representative of this group is *Mycobacterium avium-intracellulare* complex (MAIC). The Committee on *M. intracellulare* Disease of the National Consensus Conference on Tuberculosis has reported in 1985 that in parts of the U.S., pulmonary MAIC disease is as common as tuberculosis (Iseman, *et al.*, *Chest*, 87:1398, 1985). At the same time, an unprecedented increase in incidence of MAIC disease in the Acquired Immune Deficiency Syndrome (AIDS) patient population has been reported. A

1985 study (published in March 1987) by the Cleveland Clinic Foundation noted that "slightly over one-half f the MAIC isolates were from patients with AIDS" (Woods, et al., *Reviews of Infectious Disease*, 9:275,1987).

It has long been recognized that intracellular pathogens occupy a pharmacological sanctuary which often serves to protect the microorganisms from what might otherwise be an effective chemotherapeutic agent. Studies have confirmed the concept that the efficacy of certain chemotherapeutic agents in treating pathogens which can survive through intracellular colonization is related to the ability of the antibiotic to transit the host cell membrane. This problem is further complicated by the fact that drugs are normally designed to be as water soluble (hydrophilic) as possible in order to allow ready dissolution in an aqueous environment. Unfortunately, such hydrophilic drugs are often incapable of adequately traversing the host cell membrane in order to exert the chemotherapeutic effect on the intracellular pathogen. On the other hand, while lipophilic (oil-like) drugs should be more likely to be absorbed into the intracellular domain of the host cell, their lipophilic nature tends to make them insoluble such that they cannot be utilized intravenously and, as a consequence, must typically be administered in oral dosage form. Although the most convenient type of administration, oral delivery of drugs is the least efficient with respect to the amount of drug reaching the target site ("bioavailability") which may be due to poor absorption through the gut, high metabolism (inactivation) in the liver, high systemic distribution into all body tissues, or a combination of some or all of the above.

In order to overcome the problems of drug delivery associated with intracellular parasites, there has been much interest in the use of targeted, or site-directed, drug delivery systems such as liposomes. A number of publications describe the combination of lipophilic anticancer prodrugs and liposomes, mainly to reduce toxicity, prolong the circulation half-life, or inhibit rapid metabolism of the anticancer drug (Schwendener, t al., *Biochemica et Biophysica Research Communications*, 126:660, 1985; Rubas, et al., *International Journal f Cancer*, 37:149, 1986; Supersaxo, et al., *Journal of Microencapsulation*, 5:1, 1988; Matsushita, et al., *Cancer Research*, 41:2707, 1981; Hong, et al., *Journal f Medical Chemistry*, 28:171, 1985).

Pyrazinamide is a drug of moderate efficacy when administered to patients suffering from pulmonary infections. However, because it is quite toxic, pyrazinamide therapy should only be utilized if other therapy fails. In the case of pulmonary *M. avium-intracellulare* infections, the organisms reside in pulmonary alveolar macrophages such that the organism is protected from the drug which, in turn, allows the macrophages to act as a reservoir for chronic infection. Pyrazinamide is potentially more efficacious and less toxic when given in encapsulated form, for example, in a liposome, either directly to the lung via inhalation or systemically via intravenous injection. While inhalation is indicated for patients with an infection confined to the lung, intravenous injection would be the route of choice f r patients suffering from disseminated infection, for

example, patients with AIDS who are suffering from *Mycobacterium avium-intracellulare* infection. Unfortunately, drugs which are highly water soluble, such as pyrazinamide, cannot be incorporated in liposomal or emulsion drug carriers in a stable fashion for prolonged periods of time. This is due to the fact that such drugs will diffuse out of the liposome into the water phase and, in essence, exist as though the carrier were not present. This phenomenon of water soluble drug instability is known as "leakage".

As a consequence of the significant drawbacks of prior research, a strong need exists for new chemotherapeuti agents which are especially effective in the treatment of intracellular pathogenic infections. Especially useful would be a lipophilic antimicrobial drug which could be used in combination with a targeted delivery system.

SUMMARY OF THE INVENTION

Recognizing the role that intracellular pathogens play in chronic infectious diseases and the severe therapeutic limitations of pyrazinamide, the inventors produced and evaluated novel lipophilic prodrugs in an effort to develop a more effective chemotherapeutic agent. These efforts have culminated in the production and identification of new chemotherapeutic agents which are particularly effective against intracellular pathogens.

These prodrugs were developed through the lipophilic derivatization of pyrazinamide which, unlike pyrazinamide, can be stably incorporated into a targeted drug delivery system, such as a liposome. Surprisingly, these prodrugs are also capable of exerting an antimicrobial effect at much lower concentrations than seen with pyrazinamide.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

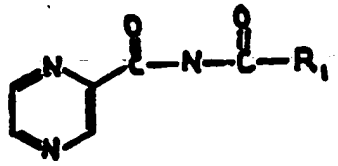
The present invention relates to novel lipophilic prodrugs of pyrazinamide which are useful in the treatment of diseases caused by intracellular pathogens. Because of their lipophilic nature, these prodrugs are particularly amenable to incorporation in a targeted drug delivery system, such as a liposome. The prodrugs comprise a core comprising pyrazinamide which is materially modified to have from about 13 to about 17 carbon atoms.

Prodrugs consist of a parent drug and a second chemical moiety covalently linked to the parent drug which provides certain desired modifications of the physicochemical properties of the parent drug. Prodrugs can also be designed to protect drugs from rapid degradation, to direct drugs to specific areas in the body, or to make drugs more hydrophilic. A prodrug can be active in itself, or be inactive and become active following chemical or enzymatic cleavage and release of the original drug.

The term "effective amount" denotes both microbicidal (killing) or microbistatic (inhibiting) activity of a compound or composition of the invention.

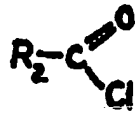
The prodrugs of the invention are effective on intracellular pathogens such as bacteria, viruses, fungi, and protozoans. These prodrugs are especially effective against bacteria of the genus *Mycobacterium*.

The lipophilic prodrugs of the invention have the formula



wherein R_1 is selected from saturated or unsaturated straight or branched chain alkyl moieties having from about 13 to about 17 carbon atoms. Preferred compounds wherein R_1 is lauroyl, myristoyl, palmitoyl, and stearoyl.

The lipophilic prodrugs of the invention can be preferably synthesized by the reaction of pyrazinamide with an acid chloride in the presence of chloroform. Preferred are acid chlorides of the formula



wherein R_2 is from about 12 to about 16 carbon atoms. Other functional groups, for example, other halogens or an anhydride, that yield the amide bond, can be substituted for chlorine to form the lipophilic acylpyrazinamide prodrug and are within the scope of the invention. Such functional groups are known or readily discernable by those of skill in the art without undue experimentation.

R_1 and R_2 are selected from saturated or unsaturated, straight or branch chain alkyl moieties. Other R_1 and R_2 groups that function equivalently to yield a lipophilic acylpyrazinamide are known to those of skill in the art and are within the scope of the invention.

The prodrugs of the invention are especially suited f r use in targetable drug delivery systems such as synthetic or natural polymers in the form of macromolecular complexes, nanocapsules, microspheres, or beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, liposomes, and resealed erythrocytes. These systems are known collectively as colloidal drug delivery systems. Typically, such colloidal

particles containing the dispersed drugs are about 50 nm - 2 μm in diameter. The size of the colloidal particles allows them to be administered intravenously such as by injection, or as an aerosol. Materials used in the preparation of colloidal systems are typically sterilizable via filter sterilization, nontoxic, and biodegradable, for example albumin, ethylcellulose, casein, gelatin, lecithin, phospholipids, and soybean oil. Polymeric colloidal systems are prepared by a process similar to the coacervation of microencapsulation.

Most preferred as a targeted delivery system for the prodrugs of the invention are liposomes. When phospholipids are gently dispersed in aqueous media, they swell, hydrate, and spontaneously form multilamellar concentric bilayer vesicles with layers of aqueous media separating the lipid bilayer. Such systems are usually referred to as multilamellar liposomes or multilamellar vesicles (MLVs) and have diameters ranging from about 100nm to about 4um. When MLV's are sonicated, small unilamellar vesicles (SUVs) with diameters in the range of from about 20 to about 50 nm are formed, which contain an aqueous solution in the core of the SUV.

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol,

phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and are saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine.

In preparing liposomes containing the prodrugs of the invention, such variables as the efficiency of drug encapsulation, lability of the drug, homogeneity and size of the resulting population of liposomes, drug-to-lipid ratio, permeability instability of the preparation, and pharmaceutical acceptability of the formulation should be considered. (Szoka, *et al.*, *Annual Reviews of Biophysics and Bioengineering*, 9:467, 1980; Deamer, *et al.*, in *Liposomes, Marcel Dekker*, New York, 1983, 27; Hope, *et al.*, *Chem. Phys. Lipids*, 40:89, 1986).

The targeting of liposomes has been classified based n anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be further distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves the alteration of the liposome by coupling the

liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposomes themselves in order to achieve targeting to organs and cell types other than the naturally occurring sites of localisation. Alternatively, liposomes may physically localize in capillary beds such as the lung or may be given by site-specific injection.

Another targeted delivery system which can be used with the prodrugs of the invention is resealed erythrocytes. When erythrocytes are suspended in a hypotonic medium, swelling occurs and the cell membrane ruptures. As a consequence, pores are formed with diameters of approximately 200-500 A which allow equilibration of the intracellular and extracellular environment. If the ionic strengths of this surrounding media is then adjusted to isotonic conditions and the cells incubated at 37°C, th pores will close such that the erythrocyte reseals. This technique can be utilized with the prodrugs of the invention to entrap the prodrug inside the resealed erythrocyte. The resealed erythrocyte containing the prodrug can then be used for targeted delivery.

The surface of the targeted delivery system may be modified in a variety of ways. Non-lipid material may be conjugated via a linking group to one or more hydrophobic groups, for example, alkyl chains from about 12-20 carbon atoms. In the case of a liposomal targeted delivery system, lipid groups can be incorporated in to the lipid bylayer of the liposome in order to maintain the compound in stabile association with the liposomal

bilayer. Various linking groups can then be used for joining the lipid chains to the compound.

Whether a ligand or a receptor, the number of molecules bound to a liposome will vary with the size of a liposome, as well as the size of the molecule, the binding of affinity the molecule to the target cell receptor or ligand, as the case may be, and the like. In most instances, the bound molecules will be present on the liposome in from about 0.05 to about 2 mol%, preferably from about 0.1 to about 1 mol%, based on the percent f bound molecules to the total number of molecules in the outer membrane bilayer of the liposome.

In general, the compounds to be bound to the surface f the targeted delivery system will be ligands and receptors which will allow the targeted delivery system t actively "home in" on the desired tissue. A ligand may be any compound of interest which will specifically bind to another compound, referred to as a receptor, such that the ligand and receptor form and a homologous pair. The compounds bound to the service of the targeted delivery system may vary from small haptens of from about 125-200 molecular weight to much larger antigens with molecular weights of at least about 6000, but generally of less than 1 million molecular weight. Proteinaceous ligands and receptors are of particular interest.

In general, the surface membrane proteins which bind t specific effector molecules are referred to as receptors. As presently used, however, most receptors will be antibodies. These antibodies may be monoclonal or

polyclonal and may be fragments thereof such as Fab, and $F(ab')_2$, which are capable of binding to an epitopic determinant.

The targeted delivery system containing the prodrug of the invention may be administered in a variety of ways to a host, particularly a mammalian host, such as intravenously, intramuscularly, subcutaneously, intraperitoneally, intravascularly, topically, intracavity, transdermally, intranasally, and by inhalation. The concentration of the prodrug will vary upon the particular application, the nature of the disease, the frequency of administration, or the like. The targeted delivery system-encapsulated prodrug may be provided in a formulation comprising other drugs as appropriate and an aqueous physiologically acceptable medium, for exampl , saline, phosphate buffered saline, or the like.

The above disclosure generally describes the present invention. A further understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE 1
SYNTHESIS OF N-ACYLPYRAZINAMIDES

Pyrazinamide (I), acetic anhydride, caproyl chloride, capryl chloride, palmitoyl chloride, and silica gel (70-230 mesh) were obtained from Sigma, St. Louis, MO. All other solvents were from Fisher, Springfield, NJ. All chemicals and solvents were of reagent grade and were used as received. N-Acetylpyrazinamide (II) was prepared according to the method of Kushner, et al. (*Journal of the American Chemical Society*, 74:3617, 1952) as illustrated in Scheme I. Purification in benzene was replaced with separation on a silica gel column. A mixture of 4 g (0.032 mol) of (I) and 20 ml (0.21 mol) of acetic anhydride was refluxed for 75 minutes at approximately 140°C. After cooling to room temperature, a precipitate consisting of unreacted (I) appeared. The precipitate was filtered off and the solution evaporated to dryness under vacuum. The residue was dissolved in chloroform and loaded onto a silica gel column (21mm x 30 cm). The column was eluted with 250 ml chloroform, followed by a 80:1 (vol/vol) mixture of chloroform/methanol. The fraction containing the pure product (II) was evaporated to dryness *in vacuo* at 70°C. The yield was 2.5g (47.3%) of a colorless solid.

N-Acylpyrazinamides (III)-(V) where prepared as noted in Scheme 2. A mixture of 200 ml chloroform and a few drops (approximately 0.25 ml) of acetylchloride were refluxed for about 30 minutes in a 250 ml three-necked round-bottom flask in order to remove moisture from the chloroform and glass surfaces. Next, 4.3 g (0.035 m l) of (I) and amounts of either caproyl chloride, capryl

chloride and palmitoyl chloride corresponding to 0.03 mol each were added and the mixture refluxed for 24 hours. While refluxing, a total of 4.74 g (0.06 mol) f pyridine was added dropwise to the solution. Upon completion of the reaction, the solution was cooled to room temperature and the needle-shaped crystals of unreacted (I) were removed by filtration. The soluti n was concentrated to approximately 20 ml by rotoevaporation and washed three times with 15 ml of cold 5% NaOH solution, followed by repeated washing with water until the wash phase was neutral. The chloroform layer was dried with anhydrous sodium sulfate. The solution was loaded onto a silica gel column and eluted with chloroform (III), chloroform/ethanol 9:1 (vol/vol) (IV), or chloroform/ethanol 8:1 (vol/vol) (V), respectively. The yields of the individual reactions were 47.3% (II), 31% (III), 25 % (IV), and 19% (V).

EXAMPLE 2

CHEMICAL ANALYSIS OF N-ACYLPYRAZINAMIDES

The structural identification data including elemental analysis, 1H-NMR and mass spectra are summarized in Table 1. Elemental analysis was performed by Atlantic Microlabs, Inc., Atlanta, GA. Proton nuclear magnetic resonance spectra were recorded on a Varian EM390 NMR spectrometer at 90 MHz. Chemical shifts were reported as parts per million (δ) relative to the internal standard tetramethylsilane. Chemical ionization high resolution mass spectra were performed on a AEI MS30 mass spectrometer. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC4 calorimeter. The HPLC set-up consisted f a Rabbit-HP pump (Rainin)

equipped with a M190 injector, a Waters model 440 absorbance detector, set to 254 nm, and a Fisher Recordall series 5000 recorder. Phosphate buffer (pH 3.4)/-acetonitril 95:5 (v/v) was used as mobile phase. An ODS (Zorbax CDS) column (4.6mmx 15cm) was employed. The flowrate was set to 1.m./min. Nicotinamide served as internal standard.

TABLE 1

STRUCTURAL IDENTIFICATION OF N-ACYLPYRAZINAMIDES

Compound	Elemental analysis %C,H,N Theoretical	^{1}H-NMR Spectral	Mass Spectra CI:m/e
II	C: 50.90 (50.91) H: 4.31 (4.27) N: 25.42 (25.44)	10.70, 9.26, 8.93, 8.75, 2.43	165.0
III	C: 59.72 (59.71) H: 6.83 (6.83) N: 18.92 (18.99)	10.42, 9.26, 8.86, 8.59, 2.31, 1.25, 0.85	221.11
IV	C: 64.86 (64.96) H: 8.39 (8.36) N: 15.05 (15.15)	10.30, 9.38, 8.76, 8.56, 2.31, 1.73, 1.25, 0.86	277.18
V	C: 69.75 (69.77) H: 9.79 (9.76) N: 11.69 (11.62)	10.50, 9.48, 8.84, 8.62, 2.30, 1.70, 1.25, 0.86	361.27

Yield, melting points and aqueous solubilities of the four products are shown in Table 2. Uncorrected melting points were determined on a Thomas Hoover capillary melting point apparatus.

TABLE 2

PHYSICOCHEMICAL PROPERTIES OF N-ACYLPYRAZINAMIDES

Compound	Yield (%)	Melting Point (oC)	Solubility (M)
I	-	189-191	1.22×10^{-1}
II	51	93-94	1.07×10^{-1}
III	≈31	78-79	6.16×10^{-4}
IV	≈25	87-87.5	1.16×10^{-5}
V	≈19	89-90	***

*** micelle formation in aqueous solution

Rf-values resulting from thin layer chromatography are shown in Table 3. Thin layer chromatography was performed on silica gel plates with fluorescent indicator (Eastman Kodak, Rochester, N.Y.) in chloroform (containing 1.1% ethanol) as mobile phase, and UV detection at 254 nm.

TABLE 3

Rf VALUES

Compound	Rf Value
I	0.27
II	0.61
III	0.71
IV	0.74
V	0.77

EXAMPLE 3

***IN VITRO* ANTIMICROBIAL ACTIVITY OF N-ACYLPYRAZINAMIDES**

Five ml Middlebrook 7H9 broth in sterile 16x100mm glass tubes with metal culture caps were inoculated with 70 ul of MAIC (strain 8T48 4/89) stock suspension having $1.55x10^7$ bacilli/ml and incubated in 5% CO_2 for 1 week in a humidified incubator. Drugs (I) - (V) were dissolved in pure ethanol to give final concentrations of 0.1, 1.0, 10 and 100 ug/ml when added to the MAIC suspension in 50 ul volumes. One untreated control and a control sample containing 50 ul ethanol without drug was included in the protocol. All samples were prepared in duplicate and incubated for 2 weeks with gentle agitation once a week. After this time, the samples were diluted 1:1000 in Middlebrook 7H9 broth and plated on Middlebrook 7H10 agar plates by placing ten 20ul drops/plate such that the total volume per plate was 200 ul. The plates were incubated for 2 weeks under conditions as described above. Colony-forming units (CFU) were counted on each side of the two halves of each duplicate plate. The results obtained are illustrated in Tabl 4.

TABLE 4

ANTIMYCOBACTERIAL ACTIVITY

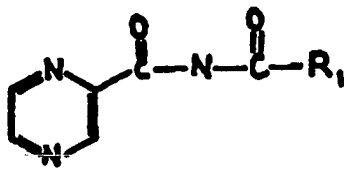
Plate	Control (no drug)	Ethanol (no drug)	(I)	(V)
1	36/39	29/36	42/39	4/3
2	36/25	49/53	48/33	0/0

As shown in Table 4, only compound V, N-palmitoyl pyrazinamide showed a significant antimicrobial effect at 100 ul/ml. Concentrations of V at 0.1, 1.0, and 10.0 ug/ml, and compounds I-IV at all concentrations tested, showed no antimicrobial effect.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made without departing from the spirit or scope of the invention.

CLAIMS:

1. A compound of the formula:



wherein R_1 is selected from saturated or unsaturated straight or branched chain alkyl moieties having from about 13 to about 17 carbon atoms.

2. The compound of Claim 1, wherein R_1 is selected from the group consisting of lauroyl, myristoyl, palmitoyl, and stearoyl.

3. The compound of claim 1, wherein R_1 is palmitoyl.

4. A antimicrobial composition comprising an antimicrobially effective amount of the compound of claim 1 together with a pharmacologically inert carrier.

5. The composition of claim 4, wherein the carrier is a targeted delivery system.

6. The composition of claim 5, wherein the delivery system is selected from the group consisting of a colloidal dispersed system and a resealed erythrocyte.

7. The composition of claim 6, wherein the colloidal system is a liposome.

8. The composition of claim 7, wherein the liposome is anatomically targeted.

9. The composition of claim 7, wherein the liposome is mechanistically targeted.

10. The composition of claim 9, wherein the liposome is passively targeted.

11. The composition of claim 9, wherein the liposome is actively targeted.

12. The composition of claim 11, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of an antibody, a sugar, a glycolipid, and a protein.

13. A method of treating an animal with a microbial infection which comprises administering to the animal a therapeutically effective amount of the compound of claim 1.

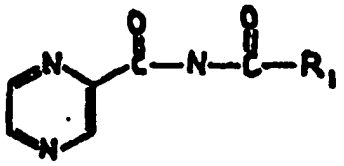
14. The method of claim 13, wherein the microbial infection is an intracellular pathogen.

15. The method of claim 14, wherein the intracellular pathogen is selected from the group consisting of a bacterium, a virus, a fungus, and a protozoan.

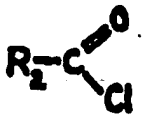
16. The method of claim 15, wherein the bacterium is by a member of the genus *Mycobacterium*.

17. The method of claim 16, wherein said bacterium is selected from the group consisting of *Mycobacterium avium-intracellulare*.

18. A process of making a compound of the formula



wherein R_1 is selected from saturated or unsaturated, straight or branched chain alkyl moieties having from about 13 to about 17 carbon atoms, which comprises reacting pyrazinamide with an acid chloride of the formula



wherein R_2 is selected from saturated or unsaturated, straight or branched chain alkyl moieties having from about 12 to about 16 carbon atoms, in the presence of chloroform and pyridine, for a time sufficient to form an acylpyrazinamide, and recovering the acylpyrazinamide.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05855

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07D 241/24, A61K 37/22, A61K 31/495, A61K 39/00
U.S.CL.: 544/406, 424/450, 514/255

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.CL.:	544/406

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CAS ON LINE

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 2,632,009 (SAFIR) 17 March 1953 See column 1.	1-3
X	S. KUSHNER ET AL, J. AMER. CHEM SOC 74, 3617 (1952) See experimental on page 3620.	18

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
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Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATI N C NTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . ... .., because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Telephone Memorandum

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

SUPPLEMENTAL SHEET

GROUPING OF INVENTION

Group I, claims 1-3 and 18, drawn to compounds and process of making classified in class 544, subclass 406.

Groups II, claims 4-12, drawn to composition formulation classified on class 424, subclass 450.

Group III, claims 13-17, drawn to method of use classified in class 514 subclass 255.

REASONS FOR HOLDING OF LACK OF UNITY OF INVENTION

The inventions listed as Groups I and II do not meet the requirements for Unity of Invention for the following reasons:

The compositions are liposomal and closely related structures and compounds which are only prodrugs may not be useful outside such special structures and thus compounds are patently distinct from the compositi ns.

The inventions listed as Groups I and III do not meet the requirements for Unity of Invention for the following reasons:

The method of use as dependent not only on compounds per se but also on particular liposomes (or similar structure) and thus compounds are patentably distinct from the method of use.